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BREAST TISSUE DOSIMETRY OF 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4,5-*b*]PYRIDINE AT HUMAN-RELEVANT EXPOSURES

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2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the most mass abundant of 19 structurally identified heterocyclic amines formed in meat by cooking under normal household conditions. PhIP is mutagenic in bacterial and mammalian cells and is a multi-organ carcinogen in rodents at high doses, causing breast tumors in female rats. Since human exposure is known to occur at mean levels of 16.64 ng/kg body-weight/day, there is concern that PhIP may be involved in human breast cancer etiology. This hypothesis is supported by the fact that mutation analysis of human breast tumors has implicated the role of chemical mutagens. Therefore, understanding the mechanisms leading to breast tumors in rats at high doses of PhIP and establishing if these mechanisms can be extrapolated to dietary levels of exposure will be useful in determining if PhIP can induce breast tumors in humans.

To study the dose-response relationships of PhIP at doses spanning several orders of magnitude, from doses producing tumors in laboratory animals to human dietary exposure levels, the technique of accelerator mass spectrometry (AMS) has been employed. AMS is an analytical technique which can detect attomole levels of ^{14}C-labeled compounds in milligram-sized biological samples, amounts impossible to detect with any other methodology. Using AMS it has been possible to study ^{14}C-PhIP tissue distribution, clearance, metabolism and covalent binding to DNA in exposed rats.

Firstly, we have established that PhIP is present in the breast tissue of lactating rats and is passed from the milk to suckling pups at doses ranging from 50-1000ng/kg body-weight. In an effort to examine potential chemopreventive therapies, results indicate that chlorophyllin treatment decreased PhIP levels in the breast tissue. Chronic feeding studies in male F344

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rats in the dose range 1mg-20ng/kg/day have illustrated that PhIP is distributed to all organs, including the breast tissue, even at the lowest dose studied. Similarly, in female F344 rats following acute doses of 5ng-100mg/kg, PhIP was detectable in the breast tissue at all doses and bound to DNA. Both tissue levels and DNA binding increased as a linear function of dose.

In addition to the use of AMS for the detection of ^{14}C, AMS methodology has now been developed to detect ^{3}H. By employing both ^{14}C and ^{3}H AMS it is possible to conduct low-level dual labeling studies, which are important in order to establish if interactions occur when multi-component exposure occurs. Rats have been administered ^{3}H-PhIP in conjunction with ^{14}C-MeIQx, another heterocyclic amine found in cooked meat. Analyses of liver and colon tissue have shown that distribution of PhIP to the liver and colon is unaffected by concurrent exposure to MeIQx.

It is our ultimate aim to develop an immunoassay to establish if PhIP-DNA adducts are formed in human breast tissue without the need to administer radioactive material. For this purpose, PhIP-DNA with a high level of modification for antibody production has been synthesized. Characterization by ^{32}P-postlabeling demonstrated that the PhIP-DNA contains 3 main adducts, the most abundant one corresponding to the dG-C8 PhIP adduct.

These studies demonstrate that PhIP is bioavailable to rat breast tissue and binds to rat breast DNA at both high and low doses. This evidence supports the hypothesis that PhIP exposure at dietary levels of exposure may contribute a breast cancer risk.

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